REMARKS

The Present Invention

Claim 1 is directed to an amplification-based method for producing a mammalian promoter-containing siRNA expression cassette.

The method comprises i) treating one strand of a double-stranded mammalian promoter sequence, in an amplification reaction mixture, with an oligonucleotide primer which is complementary to the 5′ end of the mammalian promoter sequence, wherein the mammalian promoter sequence is capable of transcribing an siRNA molecule in mammalian cells.

The method also comprises ii) treating the other strand of the mammalian promoter sequence, in the amplification reaction mixture, with a second oligonucleotide primer which is complementary to the 3′ end of the mammalian promoter sequence, wherein the second primer comprises a sequence which is complementary to a sequence encoding either a sense sequence of an siRNA molecule or an antisense sequence of an siRNA molecule, along with a terminator sequence.

The method further comprises iii) treating the amplification reaction mixture of steps (i) and (ii) in an amplification reaction at a temperature for annealing and extending said primers on the mammalian promoter sequence and at a temperature for denaturing the extension products to provide an amplified product comprising the mammalian promoter, a sequence encoding either the sense sequence of the siRNA molecule or the antisense sequence of the siRNA molecule, and the terminator sequence, and wherein steps (i)-(iii) are repeated a sufficient number of times to amplify the promoter-containing siRNA expression cassette.

Rejection under 35 U.S.C. § 103(a)

The Examiner has maintained the rejection of claims 1-9, 17, and 19-23 under 35 U.S.C. § 103(a) as being unpatentable over Shi et al. (US 2003/0180756), Medina et al. (Nucleic Acids Res 27:1698-1708, 1999), and Dietz (US 5,814,500). Applicants traverse this rejection.

The Examiner relies on Shi et al. as teaching an art-recognized goal of obtaining efficient methods for producing siRNA expression cassettes that contain a mammalian promoter (such as U6 or H1) and a termination signal sequence, wherein the expression cassettes successfully transcribe siRNAs in mammalian cells. The Examiner acknowledges that Shi et al. only teaches siRNA expression cassette (or vector) production by cloning methods.

According to the Examiner, the level and skill of an ordinary artisan were sufficiently advanced to modify the cloning-based siRNA expression cassette production method of Shi et al., thereby allowing the artisan to successfully arrive at the PCR-based amplification production of an siRNA expression cassette. Furthermore, the Examiner states that nucleic acid expression cassettes were known to be synthesized either by cloning methodology or PCR-based amplification methodology and argues that cloning and PCR-based amplification methodologies were recognized as functionally equivalent, interchangeable methodologies for producing a desired nucleic acid-based expression cassettes.

The Examiner relies on Medina et al. as teaching and suggesting that one can make an expression vector comprising a T7 promoter operably linked to a ribozyme by PCR-based amplification methods. Moreover, despite Medina et al.'s disclosure of a ribozyme expression cassette, the Examiner states that one skilled in the art would have successfully identified a primer pair that can effectively synthesize and amplify the promoter sequence that is operably linked to an siRNA (sense and antisense strands).

According to the Examiner, it would have been obvious to a person skilled in the art that making an siRNA expression cassette via PCR-amplification method would require a primer pair – one primer (oriented in a 5' direction, thus forward primer) must efficiently synthesize and amplify the nucleotide sequence of the promoter, and wherein the other primer (oriented in a 3' direction, thus reverse primer) must efficiently synthesize and amplify the nucleotide sequence that follows the promoter sequence (the siRNA sequence and the transcriptional termination signal sequence). "That is, when modifying the ribozyme expression vector production method of Medina et al. to produce an siRNA expression vector, one of ordinary skill in the art would have used a primer that 'binds at the 5' of' . . . the expression cassette that contains the promoter

sequence and another primer that 'binds at the 3' end of' . . . the expression cassette that contains the siRNA sequence and also 'binds downstream of' the siRNA sequence." Here, the Examiner relies on Medina et al.'s disclosure of PCR-amplification of a ribozyme using a primer that binds to the 5' end of a ribozyme and a primer that binds the 3' end of the ribozyme, and PCR-amplification of RNA sequences using a primer that binds downstream of the ribozyme target site. See Medina et al., p. 1700, left column.

The Examiner admits that Medina et al. does not teach using a reverse primer that binds to the 3' of the promoter sequence for producing a T7 promoter-containing expression cassette. However, according to the Examiner, it would have been obvious to one skilled in the art that the reverse primer must include a nucleotide sequence that binds at the 3' end of the U6 or H1 promoter sequence because the U6 or H1 or U1 promoter sequence is much longer than the T7 promoter sequence disclosed in Medina et al. The Examiner also argued that it would have been obvious to one of ordinary skill in the art that the reverse primer must also include a nucleotide sequence that is complementary to and binds/hybridizes to the 3' sequence of the U6 or H1 promoter sequence, **along with the siRNA sequence and the termination signal sequence**, in order to produce an siRNA expression cassette containing the full-length U6 or H1 promoter that is operably linked to the siRNA sequence and ranges about 300-500 nucleotides in length.

The Examiner also states it was known in the art that the structure of an siRNA expression cassette can successfully transcribe both the sense and antisense strands of a double-stranded siRNA in a mammalian cell.

The Examiner concludes that the combination of the prior art references teach and suggest the instantly claimed PCR-based amplification methods.

Applicants have previously argued that Medina et al. does not disclose the amplification of a promoter using a primer pair that comprises one primer that is complementary to and hybridizes to the 5' end of the promoter and a second primer that is complementary to and hybridizes to the 3' end of the promoter. Applicants have also argued that Medina et al. does not disclose the use of a primer that is complementary to and hybridizes to the 3' end of the promoter **and** further includes a siRNA sequence.

The Examiner appears to be in agreement that Medina et al. does not teach using a reverse primer that binds to the 3' end of the promoter sequence for producing a T7 promoter-containing expression cassette. The Examiner only generally relies on disclosure of PCR-amplification of RNA sequences using a primer that binds downstream of the ribozyme target site. See Medina et al., p. 1700, left column. The Examiner then argues that one skilled in the art would have used a reverse primer that binds to the 3' sequence of the promoter sequence in order to produce an siRNA expression cassette containing the full length of the longer U6 or H1 promoter.

However, the Examiner's argument that it would have been obvious to use the claimed second oligonucleotide primer because the mammalian U6 promoter or H1 promoter sequence is longer than 300 nucleotides in length is not substantiated in Medina et al. The reference teaches that the final PCR product "consists of a 988-1028 bp DNA population which contains the T7 promoter, the $tRNA_{AC}$-Rzs ($tRNA_3^{Lys}$ with random-sequence, variable-length 5' and 3' linkers connecting the ribozyme to the anticodon loop) and the ribozyme target site." See Figure 2 description. There is no suggestion in Medina et al. to forego use of the complex set of overlapping PCRs and corresponding primers to produce the 988-1028 bp product in favor of a single primer that binds to the 3' end of the promoter sequence and contains the remainder of the 988-1028 bp product, including the RNAi. Such large primers are inoperable. One skilled in the art would not have modified Medina et al. as the Examiner suggests because such a modification would render the prior art reference unsatisfactory for its intended purpose.

This also suggests that one skilled in the art would not have been motivated to combine Medina et al. with Shi et al. The overlapping PCR-amplification scheme in Medina et al. is unique to expressing a ribozyme as part of (within) the anticodon loop of tRNA without sacrificing rRNA stability or ribozyme catalytic activity. The tRNA-ribozymes were optimized to possess ideal combinations of linkers. "In $tRNA_{AC}$-Rzs, structural constraint may arise from suboptimal length of nucleotides connecting the ribozyme to the tRNA." Thus, one skilled in the art would not have combined Shi et al. with Medina et al. because one skilled in the art would

not have sought to replace the cloning system disclosed in Shi et al. with the complex PCR-scheme disclosed in Medina et al. that was unique to expressing the tRNA-ribozymes.

Furthermore, Medina et al. teaches away from use of a primer pair that comprises one primer that is complementary to and hybridizes to the 5' end of the promoter and a second primer that is complementary to and hybridizes to the 3' end of the promoter. Specifically, Medina et al. teaches the use of a primer that binds at the 5' of the $tRNA_{AC}$-Rzs which primer contains the T7 promoter sequence. Thus, Medina et al. teaches that there is no need for a pair of primers that hybridize to each end of the promoter sequence.

Applicants note that Dietz et al. is cited to show pol III promoters. Dietz et al. does not show an amplification process as claimed, and thus does not cure the deficiencies noted above.

In view of the above remarks, Applicants submit that the present invention is not rendered obvious by the combination of Shi et al., Medina et al. and Dietz et al. Withdrawal of this rejection is requested.

Conclusion

In view of the above remarks, it is believed that the claims satisfy the requirements of the patent statutes and reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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